CHANGES IN EMBRYO DEVELOPMENT, HATCHING, AND ZOEAE OF SNOW CRAB WITH VARIATION IN INCUBATION TEMPERATURE

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A

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Abstract

The effect of incubation temperature on duration of embryonic development and morphology, weight and energetic content of post-hatch zoeae was described for snow crab, *Chionoecetes opilio*, from the eastern Bering Sea held at -1, 0, 1, 3, and 6° C in the laboratory from collection to hatch. The mean incubation time increased with decreasing temperature by 32% (113 d) between 6 and -1° C. Extrusion success of females at 6° C was lower versus 0 or 3° C, but the duration of hatching did not vary significantly with incubation temperature. A one-year cycle of embryo incubation was observed, indicating that switching from one to two-year duration of embryo incubation may occur early in development. The energy content and individual weights of post-hatch zoeae were not significantly affected by temperature, indicating that longer incubation periods may not have an energetic cost. The rostro-dorsal length of zoeae incubated at 6° C was smaller than those from cooler temperatures. Conversely, the length of the 3rd abdominal somite increased significantly with decreasing temperature, perhaps serving as an indicator of incubation temperature in field collected zoeae. The consequences of varying incubation temperature appear on post-hatch zoeae appear to be limited between -1° and 6° C.

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General Introduction:

The fishery for snow crab, *Chionoecetes opilio* (Fabricius, 1788) (Brachyura: Majidae) in the eastern Bering Sea (EBS), was one of the largest and most valuable in the United States. A targeted fishery for *C. opilio* in the EBS started in the late 1970s, grew steadily during the through the 1980s, and peaked in 1991 at a harvest of 325 million pounds with a gross value of $165 million. Snow crab harvest then declined until 1996, recovered in the late 1990s, and in 2000 fell to the lowest level in over fifteen years and has continued at about this level through 2003 (Bowers 2004). This decline has resulted in increased interest in the population dynamics and reproductive biology of *C. opilio.*

Chionoecetes opilio in the northwestern Atlantic are commonly found in waters less than 3° C (Sainte-Marie and Gilbert 1998, Mallet et al. 1993). Summer trawl surveys and bottom temperature data from the EBS indicate that female *C. opilio* are found in waters that range from less than 0 to 3° C seasonally (Luchin et al. 1999; Zheng et al. 2001). These data do not include the winter months (November to February) when sea ice is at its greatest extent, and temperatures are typically colder. In the Gulf of St. Lawrence, Canada, *C. opilio* are closely associated with a low temperature water mass, the Cold Intermediate Layer (CIL) (Sainte-Marie and Gilbert 1998; Lovrich et al. 1995; Mallet et al. 1993; Thompson and Hawryluk 1989). The core temperature of the CIL is typically between –1 and 1° C with interdecadal changes in core temperature of up to 2° C (Sainte-Marie and Gilbert 1998). The temperature of the cold pool (a water mass similar to the CIL) in the Bering Sea is more variable interannually, but has a core

temperature that varies between 0° and 3° C, though temperatures as cold as -1.2° C have been recorded (Luchin et al. 1999; Azumaya and Ohtani 1995).

A. General Biology of *Chionoecetes opilio*

Complex dynamics in mating, reproduction, growth, and movement, are features of *C. opilio* life history and biology. Temperature directly or indirectly affects many aspects of their life history. Therefore, a general understanding of their life history is crucial to understanding the potential effect of temperature on *C. opilio*.

I. Distribution

Chionoecetes opilio have a circumpolar distribution in both the Atlantic and Pacific Oceans and can be found in the Sea of Japan, Sea of Okhotsk, Bering Sea, Chukchi Sea, Arctic Ocean (Jadamec et al. 1999; Zheng et al. 2001), in the northwestern Atlantic Ocean from the Gulf of Maine to Greenland (Pohle 1991), and were recently reported as an invasive species in the Barents Sea (Kuzmin 2000). *C. opilio* are usually found in benthic mud or sand habitats at depths 60 to 400 m. (Elner and Beninger 1995). Early benthic stages of *C. opilio* are distributed in discrete temperature strata; their distribution varies by instar and is more dependent on temperature than substrate preferences (Dionne et al. 2003). In the Gulf of St. Lawrence on the eastern coast of Canada, *C. opilio* are found mainly in areas with muddy substrate at temperatures from -1 to 2° C (Lovrich et al. 1995). In the southeastern Bering Sea, *C. opilio* are most often found at depths of 50 to 150 m along the middle of the continental shelf where bottom water is the coldest (Zheng et al. 2001).

Chionoecetes bairdi, Tanner crab, is a congener of *C. opilio* but lives in warmer water at depths greater than 100 m along the outer continental shelf in the southeastern Bering Sea. *Chionoecetes bairdi* are found along the west coast of North America from Oregon north to the Gulf of Alaska and Bering Sea (Jadamec et al. 1999). In areas of overlap, the two species interbreed producing a hybrid (Merkouris et al. 1998).

Over the last two decades the spatial distribution of both male and female *C. opilio* in the EBS has moved from southeast to northwest (Orensanz et al. in press 2005, Zheng et al. 2001). A climate-driven regime shift is hypothesized as a possible cause for this shift (Zheng et al. 2001), in which the Aleutian Low Pressure System intensified in strength from 1977 to 1988. Over the last few decades *C. opilio* concentrations have moved to colder northwest waters, possibly to avoid warmer waters to the south. In the EBS, *C. opilio* moved in a southwesterly cross-shelf direction as their ontogeny progresses possibly in response to gradients in near bottom temperature or depth (Ernst et al. 2005; Zheng et al. 2001).

II. Predation

As an abundant and widely distributed species, *C. opilio* is not only the target of a large commercial fishery, but also serves as a significant prey source for at least four species of marine mammals (spotted seals, bearded seals, Pacific walrus, and sperm whale), seven species of invertebrates, and 26 species of fish (Jewett 1982). In the southeastern Bering Sea *C. opilio* are subject to heavy predation by skates (*Raja spp.*), Pacific cod (*Gadus macrocephalus*), the wattled eelpout (*Lycodes palearis*), and four assorted sculpins (*Cottidae*) (Jewett 1982). The most common invertebrate consumer of

Chionoecetes crabs are other *Chionoecetes* crab, both conspecifics and congeners (Jewett 1982). Predation by Pacific cod is severe enough to potentially negatively affect the strength of the age-1 and age-2 year classes for *C. opilio* (Zheng and Kruse 2000).

Density-dependent cannibalism has been studied as a possible mechanism of recruitment regulation in *C. opilio.* In the northwestern Gulf of St. Lawrence, 7% of *C. oplio* overall and up to 22% of 45-55 mm carapace width (CW) males were found with conspecifics in their stomach contents (Lovrich and Sainte-Marie 1997). Immature crabs (30-37 mm CW) are most vulnerable to predation, which is primarily caused by adult males (Dutil et al. 1997).

III. Recruitment

As is typical of other crustacean populations, the abundance of *C. opilio* in both the northwestern Atlantic and North Pacific oceans is highly variable due to variation in recruitment. An eight-year cycle of variation in recruitment has been observed in the northwestern Gulf of St. Lawrence (Sainte-Marie et al. 1996) and is characterized by five years of medium to strong recruitment followed by three years of weaker recruitment (Sainte-Marie and Gilbert 1998). Recruitment patterns and stock-recruitment relationships for EBS snow crab are similar to those from the Gulf of St. Lawrence with cycles of strong and weak recruitment occurring every few years and cyclic patterns in spawning biomass (Zheng and Kruse 2003).

IV. Maturity

Age and size at sexual maturity are variable for both male and female *C. opilio.* Age and size at maturity are affected by temperature. Small decreases in molt increment

and marked increases in intermolt period occur with decreasing temperature from 4 to -1° C, so that at a given age, a crab growing in warmer waters is larger than a counterpart growing in colder water (Sainte-Marie and Gilbert 1998). Male *C. opilio* have a similar life history to females through the first five instars with molts occurring on a biannual basis, and later molts occurring on an annual basis (Comeau et al. 1998a). Early reports indicated very divergent values for the age of male *C. opilio* recruiting to the minimum legal size limit (95 mm CW) in the Gulf of Saint Lawrence fishery with values ranging from 4.5 years to 9.5 years (Sainte-Marie et al. 1995). More recent studies have placed the age of entry into the fishery at 8.7 (Sainte-Marie et al. 1995) and 9 years (Comeau et al. 1998a). The minimum legal size limit for male *C. opilio* in the EBS fishery is 78.7 mm CW (Bowers 2004). After much debate on the terminal molt hypothesis for male *C. opilio*, several investigators decided that a terminal molt to maturity for males occurs in the Gulf of St. Lawrence and Japan (Comeau et al. 1998a; Elner and Beninger 1995; Sainte-Marie et al. 1995; Sainte-Marie and Hazel 1992) and EBS (Tamone et al. 2005).

V. Mating

Accurate knowledge of mating systems used by *C. opilio* is critical to management of this species. *Chionoecetes opilio* exhibits complex mating behavior that varies with reproductive status, availability of mates, and mating history. Females reproduce either biennially or annually depending on water temperature (Moriyasu and Lanteigne 1998). Both polyandrous and polygynous mating behaviors are found in *C. opilio*. Individual females mated with multiple males in the laboratory (Sainte-Marie et al. 1999; Urbani et al. 1998; Sainte-Marie and Lovrich 1994), and the spermatheca of

females captured in the wild can contain as many as 10 to 12 ejaculates (Sainte-Marie et al. 2000). Male *C. opilio* from the Gulf of St. Lawrence have competitive interactions between males with large chelae who have teminally molted (TM) and sexually mature males who are non-terminal molt (NTM) and have smaller chelae, to grasp and guard virgin females through the primiparous molt and then mate with them (Sainte Marie et al. 1999). The amount of time spent and success of guarding varies with the ratio of males to females, the maturity (completion of terminal molt) status of males, and with male CW (Rondeau and Sainte-Marie 2001; Sainte-Marie et al. 1999; Sainte-Marie et al. 1997). Terminally molted males are more successful at guarding and mating with females than their NTM counterparts. They are also more able to take females away from NTM males (Sainte-Marie et al. 1997). Assortative mating occurred in the GSL with males that had more limbs, greater carapace width, and greater shell hardness successfully grasping nulliparous females (molted to maturity, but not having extruded eggs) and pubescent females who were near their molt to maturity (Sainte-Marie et al. 1999). The amount of time spent guarding females prior to mating and the amount of sperm transferred at mating varies with the ratio of males to females. In treatments of 2:20 and 6:20 males/females the average times guarded per female were 2.9 days and 5.6 days, respectively; larger CW males guarded females longer, and were thought to have expended more sperm than their smaller counterparts (Rondeau and Sainte-Marie 2001). All the females in each treatment were inseminated. However, a male would practice sperm economy giving less sperm per female in order to inseminate all available females, even if the amount of sperm transferred was below the threshold needed to fertilize an

entire clutch (Rondeau and Sainte-Marie 2001). In noncompetitive mating, smaller males (40-60 mm CW) mated repeatedly for shorter periods of time while large males (120-140 mm CW) copulated just once for a longer period of time (Sainte-Marie et al. 1997). While NTM males have mated successfully with multiparous females in the laboratory, intraspecific competition with TM males in the wild can exclude them from mating (Comeau et al. 1998b). Similar bipartite breeding patterns are also known for *C. opilio* from the EBS (Somerton 1981). The precopulatory embrace has been observed to last up to eight days in the laboratory (Somerton 1981).

Polyandrous mating behavior raises the question of whether the sperm of a single male or multiple males fertilize a single clutch. Thirty-two of 34 primiparous females isolated after hatching their first clutch extruded a second clutch that was fertilized with sperm held through at least one reproductive cycle (Sevigny and Sainte-Marie 1996). For both wild-caught and laboratory- mated females from the Gulf of St. Lawrence, genetic testing showed that the last male to mate before ovipostion gained paternity of the entire clutch (Urbani et al. 1998; Sevigny and Sainte-Marie 1996). Female *C. opilio* can mate one or more times after extruding their eggs, and sperm can be stored and used to fertilize subsequent clutches of eggs (Urbani et al. 1998; Sainte-Marie et al. 1997). Sperm viability in *C. opilio* has not been closely examined, but for *C. bairdi*, the viability of sperm decreased over time, and some multiparous females either did not spawn or extruded a clutch of unfertilized eggs when they were isolated from mating (Paul 1984).

VI. Fecundity and Growth

Few studies have estimated fecundity for *C. opilio*. These estimates vary with geographic region and are positively correlated with increasing CW and reproductive status (Jewett 1981; Sainte-Marie 1993). Fecundities of *C. opilio* collected from the Chukchi Sea were compared with fecundities for *C. opilio* from the southeastern Bering Sea and the Gulf of St. Lawrence (Jewett 1981). Chukchi Sea females had fecundities in the range of 12,900 to 37,100 eggs per clutch for females ranging from 40 to 64 mm CW. Fecundities for southeastern Bering Sea females ranged from 28,200 to 74,800 (50-79 mm CW), and Gulf of St. Lawrence fecundities were 31,800 to 114,900 (50-89 mm CW). Primiparous females from the Gulf of St. Lawrence were 77.3% to 83.6% as fecund and had larger egg diameters (0.7 – 1.4%) than multiparous females (Sainte-Marie 1993). The majority (70.2%) of female *Chionoecetes japonicus*, a commercially important, deep-water congener of *C. opilio* from the Sea of Japan, had fecundities in the range of 10, 000-40,000 eggs. A significant ($r=0.38$, $P<0.01$) but weak correlation between fecundity and maternal carapace width was observed (Yosho 2000).

VII. Life History

Duration of incubation for *C. opilio* varies with temperature and reproductive status (Rugolo et al. 2004; Lovrich et al. 1995; Sainte-Marie and Hazel 1992). In the northwestern Atlantic, primiparous females have been observed to mate and extrude eggs in February in shallower waters compared to multiparous females that extrude from April to June (Lovrich et al. 1995). Diapause, a period of arrested development by which embryos can be held at a given developmental stage for a variable amount of time,

extends the incubation period of *C. opilio* at low temperatures (Moriyasu and Lanteigne 1998). Both captive and wild females from the Gulf of St. Lawrence in colder (-1 to +1° C) water had a two-year embryo development cycle with two distinct periods of diapause, with the first diapause at egg stages 3 or 4 (cleavage and blastula, gastrula) lasting about six months and the second diapause at stages 11-12 (eye-pigment formation, chromataphore formation) lasting about four months (Moriyasu and Lanteigne 1998). The proportion of crabs on a two-year versus one-year cycle is unknown (Moriyasu and Lanteigne 1998) and relates to interannual fluctuations in the temperature of the CIL (Sainte-Marie and Gilbert 1998).

Laboratory experiments and long-term field studies confirmed the presence of temperature dependent switching in the duration of embryonic development from a one year (annual) to two years (biennial) cycle for a cold-water majid (*Hyas araneus*) from the Barents Sea (Petersen 1995), *C. opilio* females in the EBS (Rugolo et al. 2004) and a *C. opilio* population in the northwest Atlantic (Sainte-Marie and Gilbert 1998). *H. araneus* (Brachyura: Majidae), endemic around Helgoland Island, Germany were found to switch from a two to a one-year cycle between 12 and 6° C (Petersen 1995). In Baie Sainte-Marguerite, Canada, *C. opilio* egg development has been monitored since 1990, and switching from a two- to one-year cycle of embryo incubation was observed in association with a warm winter and a warming trend in the CIL (Sainte-Marie and Gilbert 1998). A two-year cycle of embryo incubation has been found in both primiparous and mulitparous females at temperatures below 1° C in the EBS (Rugolo et al. 2004, Rugulo, pers. comm.). A two-year cycle of embryo incubation has also been observed for

Chionoecetes japonicus (a congener of *C. opilio* distributed at greater depths), from the Sea of Japan, which occupy habitats with stable water temperatures between 0.15 to 0.18 °C (Yosho 2000).

C. opilio larvae hatch as a prezoea and go through two zoeal stages followed by a megalopa stage. The prezoeal stage is covered by an embryonic cuticle without any spines or processes (Motoh 1982). Within hours after hatching, this cuticle is shed, and the larvae becomes a stage I zoea (Haynes 1973; Motoh 1973; Wencker et al. 1982). The zoea I stage lasts between 20 and 35 days before molting to a stage II zoea (Lovrich and Oullet 1994; Davidson and Chin 1991; Incze et al. 1982). The duration of the larval stages is dependent on temperature, with warmer temperatures causing shorter intermolt intervals and shorter planktonic period (Davidson and Chin 1991; Wencker et al. 1982). The total planktonic period, estimated in the laboratory for *C. opilio* was 71 days with a mean temperature of 8.2° C (Davidson and Chin 1991) and was predicted to be about 90 days for *C. opilio* in the EBS (Incze et al. 1982). Zoeae are planktivorous (Kon 1979) and euryhaline, being able to withstand wide ranges of salinity (LS 50 of 10 to 42 ppt for 24 h at 14° C) (Charmentier and Charmantier-Duares 1995).

After the megalopa stage, crabs molt to the first benthic instar, which is the first stage that appears similar to adults (Adams 1979). In Bonne Bay, Newfoundland, Canada, *C. opilio* progress through juvenile instar stages in 2 to 2 ½ years. The molting frequency of females is twice a year, up to instar V, and annually thereafter (Comeau et al. 1998a). While a few females may reach their terminal molt to maturity at instar X (seven years old), most females achieve their terminal molt to maturity at 8 years (instar

XI) with an average carapace width of 65.4 mm. The life expectancy of a female, estimated by a size-frequency distribution from Bonne Bay, Newfoundland, is 13 years (Comeau et al. 1998a). The accumulation of the morphological pigment lipofuscin was used to generate age estimates that range from 2.4 to 6.8 years for new-shell and 4.5 to 7.7 years for old-shell mature females from the EBS (Bluhm and Shirley in press 2005). Both of these studies suggest that females live up to a maximum of five years after terminal molt, and thus on a two-year reproductive cycle, a female would bear only one to two clutches of eggs in her lifetime.

VIII. Physiology and Energetics

Specialized physiological and energetic strategies allow *C. opilio* to live at cold temperatures. Individuals at -1° C weighing more than 200 g. wet weight had adequate food intake to meet metabolic needs, but below this threshold were in negative energy balance (Thompson and Hawryluk 1989). Oxygen uptake, food consumption, and activity in were measured in TM male *C. opilio* (Foyle et. al. 1989). Temperatures of 12 to 15° C were found to be lethal, and though oxygen uptake increased slightly with temperature, metabolic requirements could be met at lethally warm temperatures. Above 7° C metabolic costs surpassed caloric intake, and above 12° C animals would not feed at all. Spontaneous activity levels peaked at a temperature of 0° C. When variables were combined in an overall growth equation, a negative growth rate was predicted below 1° C, which is surprising since *C. opilio* are commonly found at these temperatures in the northwestern Atlantic and EBS. The effect of temperature during starvation on *C. opilio* has also been tested for hard-shell male adult *C. opilio* from the Gulf of St. Lawrence

which were starved while being held for 91 days. Mortality increased with holding temperature; with 7.1%, 12.9%, and 20.7% mortality respectively at 1, 5, and 10 °C (Hardy et al. 2000).

Temperature affects the growth, movement, distribution, at of *C. opilio* at various stages of development with temperature dependent switching from a one-year to a two-year cycle of embryo incubation one of the most striking effects. The occurrence of a two-year cycle for *C. opilio* in the EBS has been confirmed by field studies (Rugolo et al. 2004), and this thesis addresses the duration and patterns of embryonic development at discrete temperatures and possible consequences of variation in embryonic development for post-hatch zoeae. The following hypotheses are proposed to examine the relationship of the duration of embryonic development with temperature and whether these differences have consequences for post-hatch *C. opilio* zoeae.

B. Hypotheses

I. Embryonic Development

H_0: There is no change in the duration of embryonic development with temperature.

H_a: The duration of embryonic development changes with incubation temperature.

II. Larval Morphology

H_0: The spine lengths of stage-one *C. opilio* zoeae do not vary with embryonic incubation temperature.

H_a: The spine lengths of stage-one *C. opilio* zoeae vary with embryonic incubation temperature.

III. Larval Energy Content

H_0: The energetic content of stage-one *C. opilio* zoeae do not vary with embryonic incubation temperature.

H_a: The energetic content of stage-one *C. opilio* zoeae vary with embryonic incubation temperature.

IV. Larval Weight

H_0: The individual weights of stage-one *C. opilio* zoeae do not vary with embryonic incubation temperature.

H_a: The individual weights of stage-one *C. opilio* zoeae vary with embryonic incubation temperature.

Chapter 1 addresses the effect of incubation temperature on embryonic development, hatching, and spawning, and Chapter 2 addresses the effect of incubation temperature on characteristics of post-hatch zoeae.

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Chapter 1

Changes in embryonic development and hatching in the snow crab, *Chionoecetes opilio*, from the eastern Bering Sea with variation in incubation temperature[1]

[1] Joel B. Webb, Ginny L. Eckert, Thomas C. Shirley, and Sherry L. Tamone. Journal of Crustacean Biology. To be submitted.

1.1 Abstract

Declines in catch and abundance of the snow crab, *Chionoecetes opilio,* in the eastern Bering Sea have prompted interest about the life history of this species. Water temperature in the eastern Bering Sea affects adult *C. opilio* distribution and movement, and below 1° C females appear to require two years for embryonic development. However, little is known about the timing or mechanism for the switch from a one-year to two-year cycle or how temperature affects embryo development or larval release. In this study ovigerous female *C. opilio* were collected by trawl from the eastern Bering Sea in July 2002, transported to Juneau, and held at five temperatures (-1, 0, 1, 3, and 6° C) in the laboratory from egg stage five to hatching. The duration of incubation increased with decreasing temperature by 108 d, however a switch to two-year duration of embryo incubation was not observed. A short diapause period in embryo development was observed females held at 3 and 1° C but not in egg masses held at -1, 0 or 6° C. The duration of hatching was not significantly affected by temperature. Successful extrusion of a new clutch following larval release was much lower at 6° C, suggesting that ovarian maturation processes may also be thermally influenced. Our study demonstrates that a switch from one-year to two-year reproduction cannot be triggered by changing the thermal regime after several months of embryonic development. Ontogenetic migration of females from colder to warmer waters during early development may be important for increasing population reproductive potential by maintaining a one-year reproductive cycle. Further research into female movements and potential links with environmental

temperature and embryonic development could clarify the importance of this apparent relationship.

1.2 Introduction

Snow crab, *Chionoecetes opilio* (Fabricius, 1788) (Brachyura: Majidae), are found in high latitude, cold-water, continental shelf environments throughout much of the northern hemisphere with commercial fisheries occurring in the Northwest Atlantic, Sea of Japan, and eastern Bering Sea (EBS). Declining catch, catch per unit effort, and value of the eastern Bering Sea fishery (Figure 1.1) have led to the listing of the EBS *C. opilio* population as "overfished" under the Magnuson-Stevens Fisheries Conservation and Management Act (National Marine Fisheries Service, 1999).

Warmer than average water temperatures have predominated in the Bering Sea since 1977 when a regime change from colder to warmer ocean conditions occurred (Hunt *et al.*, 2002). Since this time changes in EBS water temperature and ice cover have altered the ecosystem structure, biogeographical distribution, and life histories of organisms in the region (Overland *et al.*, 2005; Overland and Stabeno, 2004; Hunt *et al.*, 2002). Temperatures in the EBS have increased as much as 2° C in the past decade with warm temperature anomalies arriving earlier in the spring and persisting longer into the fall than in past years (Overland and Stabeno, 2004). Warm temperatures are likely to continue in the EBS in the near future with cold-water biomes continuing to expand northward (Overland *et al.*, 2005).

The movement, physiology, early life history, and reproductive potential of female *C. opilio* are all affected by temperature. Changes in distribution and abundance

of EBS *C. opilio* have been observed over the last several decades, when the centers of distribution of both mature male and female *C. opilio* shifted from southwest to northeast in the EBS from the late 1970s to the late 1990s (Ernst *et al.*, 2005; Orensanz *et al.*, in press 2005; Zheng *et al.*, 2001). This shift in distribution may have been associated with the regime shift in physical oceanography and climate in the EBS starting in 1976-1977 (Zheng *et al.*, 2001). Large scale movements of female *C. opilio* in the EBS are likely in response to temperature. The northward displacement of the southern boundary of the EBS cold pool (<2° C) was followed by a northward shift in the distribution of mature females with a six year lag time (Orensanz *et al.*, in press 2005). Subsequent southward range expansion of *C. opilio* may be limited by predation (Orensanz *et al.*, in press 2005) from juvenile Pacific cod (*Gadus macrocephalus*) that prey heavily on early benthic instars of *C. opilio* (Livingston, 1989), because Pacific cod and similar species are likely to increase in abundance and expand northward with warmer seawater temperatures in the EBS (Hunt *et al.*, 2002). Smaller scale movements of female *C. opilio* in the EBS are also influenced by temperature. Females are hypothesized to follow gradients in near bottom temperature or depth from colder to warmer areas, undergoing a unidirectional offshore ontogenetic migration oriented from northeast to southwest over a distance of greater than 140 km (Ernst *et al.*, 2005).

Temperature affects *C. opilio* physiologically throughout their life history. As a stenothermic species, adult *C. opilio* are energetically confined to waters below 7° C, with peak activity occurring at 0° C (Foyle *et al.*, 1989). For early benthic instars, temperature rather than substrate determines distribution, and instars segregate among

discrete temperature strata (Dionne *et al.*, 2003). The duration of embryonic incubation in *C. opilio* can be extended by small changes in temperature. Primiparous and multiparous females have reproductive cycles of 18 and 12 months respectively in warmer (3 to 5° C) water and 27 and 24 months at colder (-1 to +1° C) temperatures (Comeau *et al.*, 1999; Moriyasu and Lanteigne, 1998; Mallet *et al.*, 1993; Sainte-Marie, 1993; Watson, 1970) in the Gulf of St. Lawrence, Canada (GSL). Recent field studies have confirmed the presence of a two-year cycle of embryonic development for both primiparous and multiparous *C. opilio* in the EBS at temperatures below 1° C in the northern portions of their range (L. Rugulo, NOAA, NMFS, Kodiak, Alaska pers. comm.). The potential range of temperatures experienced by mature female *C. opilio* in the EBS is approximately -1 to 3° C (Zheng *et al.*, 2001; Luchin *et al.*, 1999), with females in the northern portion of the range more likely to experience colder temperatures for longer periods of time, as temperature generally increases with decreasing latitude in the EBS (Luchin *et al.*, 2002).

The presence of a two-year reproductive cycle could limit the reproductive potential of *C. opilio* in both the EBS and GSL. In the GSL, female *C. opilio* undergo a terminal molt to reproductive maturity at 5.5 to 6.5 years of age (Alunno-Bruscia and Sainte-Marie, 1998) and have a post-terminal molt survival of five to six years (Sainte-Marie, 1993), which on a two-year cycle may produce only two clutches during a lifetime (Sainte-Marie, 1993). In the EBS, post-terminal molt survival for female *C. opilio* has been estimated at six to seven years based on instar analysis (Ernst *et al.*, 2005) and two to four years based on lipofuscin accumulation (Bluhm and Shirley, in press 2005), and

as a result, female *C. opilio* in the EBS are likely to produce only two to three clutches in a lifetime.

The effect of temperature on the duration of embryonic development has been well described for *C. opilio* from the northwestern Atlantic (Comeau *et al.*, 1999; Moriyasu and Lanteigne, 1998; Mallet *et al.*, 1993; Sainte-Marie, 1993). However, little is known about the effect of temperature on the duration of embryonic incubation, patterns of embryo development, or hatching of *C. opilio* from the EBS. Substantial decreases in the rate of embryo development in decapod crustaceans, including *C. opilio,* are due to periods of diapause or suspension of growth (Moriyasu and Lanteigne, 1998; Wear, 1974). On a two-year reproductive cycle, *C. opilio* from the GSL have two distinct periods of diapause during embryonic development. The first diapause period occurs at the gastrula stage (stage 4) and lasts for six months, and the second is at the eye pigment formation stage (stage 11) and lasts for three to four months (Moriyasu and Lanteigne 1998). The presence or length of diapause periods in the embryo development of *C. opilio* from the EBS is unknown.

The purpose of this study is to examine the effect of a range of environmentally relevant incubation temperatures on the duration and rate of embryonic development, duration of hatching, and extrusion of a subsequent clutch following larval release for multiparous female *C. opilio* from the EBS.

1.3 Methods and Materials

Ovigerous females were collected by bottom trawl on July 5, 2002 south of St. Matthew Island (58° N, 173° W) in the EBS at a depth of 117 m with a bottom

temperature of 3.02° C and transported by air to Juneau, AK on July 10, 2002. In Juneau, crabs were maintained in 700 L flow-through seawater tanks cooled by chillers (Frigid Units™ Inc. Model D1-33) to constant temperature. Temperatures were monitored using Stowaway Tidbit™ temperature loggers at 15 m intervals. Twenty-five females each were assigned to treatments of -1, 0, 1, 3 and 6 °C in a random stratified design based on carapace width such that no significant differences in crab sizes existed among treatments. Mean tank temperatures °C (± 1SD) averaged daily over the duration of the study for the 6, 3, 1 0 and -1 °C treatments were 6.32 (±0.36), 3.24 (±0.18), 1.37 (±1.03), 0.12 (± 0.94), and -0.89 (± 0.59), respectively. Females that were primiparous, had low clutch fullness scores, or had two or more missing or damaged limbs were excluded from the study. All statistical analyses were conducted using SAS release 8.02 (SAS Institute, Cary, NC). All averages are reported as mean ± SD.

1.3.1 Embryonic Incubation and Development

Approximately 50 embryos were collected and preserved in Bouin's solution at four week intervals from each of 15 females per tank. Embryos were consistently collected from an area near the center of the clutch to control for potential variation in developmental stage within the clutch. Within a few days of collection, 10 preserved embryos per female were staged to the nearest of 14 developmental stages using criteria established for the embryonic development of *C. opilio* by Moriyasu and Lanteigne (1998) (Table 1.1). Embryonic development is a continuous process but staging of embryos is based on static morphology of the embryo when certain physiological and morphological features are observable through the course of development (Moriyasu and

Lanteigne, 1998). The developmental stage of the clutch was assigned for each female at each sampling date using the mean developmental stage observed among the 10 embryos. Little variation in stage was observed among sampled embryos from the same clutch for a given sampling date.

1.3.2 Hatching

When embryos neared the final developmental stage, tanks were monitored daily for the presence of larvae by placing filters on tank outflows. When hatching commenced seven females with a representative range of carapace widths from each tank were placed in plastic boxes (33 x 18 x 10 cm) with sides replaced by 500 μm (47% open) Nitex™ mesh to allow adequate seawater circulation while retaining larvae hatched by the female. All zoeae were removed from the container at 24-h intervals, and the number of zoeae hatched was estimated on an order of magnitude scale (0-10, 10-100, 100-1000, 1000+). Duration of hatching was measured from the first release of 10 or more zoeae in a 24 h period to the final release of ten or more zoeae in 24 h. Females were kept in enclosures either until spawning of a new clutch or for at least two weeks after the conclusion of hatching. Differences in time from collection to hatch among temperature treatments were examined using one-way ANOVA with Tukey-Kramer multiple comparisons tests.

1.4 Results

1.4.1 Duration of Embryonic Incubation

The duration of embryonic incubation from field collection to larval release increased significantly with decreasing incubation temperature ($F_{4, 28}$=167.25, $p<0.0001$),

except 0° and 1° C, which were not significantly different from each other ($p<0.05$) (Figure 1.2). The mean incubation time from collection to first hatch (days ± SD) was 240 ± 10 d at 6° C, 276 ± 8 d at 3° C, 314 ± 8 d at 1° C, 331 ± 11 d at 0° C, and 353 ± 2 d at -1° C (Figure 1.2). Mean incubation time decreased 113 d (33%) between -1 and 6° C.

1.4.2 Embryonic Development

The rate of embryonic development increased with increasing temperature (Figure 1.3). Embryonic development at 6° C (6 – 8 months) was half that of embryos at -1° C (13 – 14 months). Embryos at 6° C reached the final stage of development between January and March 2003, while embryos from the 3, 1, 0, and -1° treatments reached the final stage in March, April, May, and August respectively. Differences in rates of development were observed as early as five weeks after placement in treatments, when embryos at 6° C had progressed one to two egg stages further than those at -1 to 1° C (Figure 1.4). A two-month diapause period, noted by a decrease in mean developmental rate was evident at stages 12 or 13 in the 3 and 1° C treatments. Developmental trajectories were largely linear (Figure 1.3), with a slope of 0.835 ($R^2 = 0.99$) at 6° C, 0.90 ($R^2 = 0.99$) at 3° C, 0.87 ($R^2 = 0.98$) at 1° C, 0.80 ($R^2 = 0.98$) at 0° C, and 0.594 ($R^2 = 0.98$) at -1° C. A 28.9% reduction in developmental rate (slope) with temperature between 6 and -1° C was observed.

1.4.3 Hatching and Spawning

The mean duration of hatching was 11 d for all females with minimum of 7 d and a maximum of 17 d to complete hatching (Figure 1.5). No significant differences in the

duration of hatching ($F_{4, 19}$=0.39, p<0.863) were observed among temperature treatments. No hatching was observed at -1° C due to high maternal mortality in this treatment.

The number of females that spawned a new clutch subsequent to completion of hatching varied among temperatures. Zero (0%) of seven females that completed larval release (eclosion) at 6° C spawned a new clutch within two weeks. Six (86%) of seven females at 3° C and all (100%) five females that completed larval release at 0° C spawned a new clutch. Comparisons with 1° and -1° C treatments were not possible due to high maternal mortality prior to spawning at those temperatures.

1.5 Discussion

The length of embryonic incubation increased with decreasing temperature for *C. opilio*. An increase in embryonic incubation of 17 days per 1° C occurred at temperatures (3, 1, and 0° C) likely to be experienced by female *C. opilio* in the EBS. This result is similar to an increase of 21.4 days per 1° C in the total duration of incubation for primiparous *C. opilio* from the GSL (Moriyasu and Lanteigne, 1998). Differences in these values are likely due to longer overall incubation periods for primiparous versus multiparous females (Sainte-Marie, 1993), however direct comparisons are not possible because data on changes in development time with temperature are not available for multiparous females from the GSL nor primiparous females from the EBS. Another difference between these two studies is that females in our study were held at constant temperature from collection at two to three months post-extrusion to hatch, while females from the GSL (Moriyasu and Lanteigne, 1998) were held at constant temperature for the duration of the reproductive cycle (embryo extrusion to hatch).

A two-year cycle of embryonic development was not observed in our study. Based on temperature records from NMFS summer trawl surveys and the temperature recorded during collection, the previous thermal history of our females was likely ~ 2 - 3° C, which is warmer than the temperature (<1° C) at which *C. opilio* both in the EBS (L. Rugulo NOAA, NMFS, Kodiak, Alaska pers. comm.) and the GSL (Moriyasu and Lanteigne, 1998) switch to a two-year cycle of embryo incubation. Our results indicate that changing temperature later in development (two to three months post-extrusion) does not induce a two-year incubation period. Embryos from our lab study sampled in early September were at the prenaupliar/nauplius stage (stage 6 to 7) at -1° C and at the metanauplius stage (stage 9) at 6° C. According to Moriyasu and Lanteigne (1998), the clutches of females on a two-year reproductive cycle from the GSL would be at the blastula or gastrula stage (stages 3-4) in the early fall, having arrested development for 6 months at stage 4. It is likely that our females skipped this first diapause period due to warmer temperatures earlier in embryonic development. Female *C. opilio* in the EBS follow gradients of near-bottom temperature from colder (~ 0° C but interannually variable) (Luchin et al. 1999) to warmer waters (< 2° C) as they migrate offshore in a southwesterly direction during the year following the primiparous molt (Ernst et al. 2005). This migration of females from colder to warmer waters in the EBS before extrusion of the multiparous clutch could increase the reproductive potential of the population by increasing the proportion of the population on a one-year versus two-year reproductive cycle if females are in waters warmer than 1° C during the first several months post-extrusion.

The presence or absence of a diapause or resting period is the primary cause of variation in the length of the embryonic incubation cycle in crustaceans (Wear 1974). Two periods of diapause occur in the development of *C. opilio* embryos from the GSL held at 1.85° C on a two-year reproductive cycle. The first diapause period occurs at the gastrula stage (stage 4) and lasts for six months, and the second is at the eye pigment formation stage (stage 11) and lasts for three to four months (Moriyasu and Lanteigne 1998). We observed a two-month diapause in some (3 and 1° C), but not all, temperature treatments at the reduced yolk stage (stage 13). Diapause was not apparent in our study during the development of embryos at 0 or -1° C; the extended duration of embryonic development at these temperatures was due to an overall decrease in developmental rate rather than periods of diapause (Figure 1.3). Wear (1974) has noted that changing temperatures during diapause does not change the length of resting period, but rather the rate of development responds to increased temperature after the diapause period. Our results indicate that the presence/absence of diapause periods, the timing of diapause induction, and the duration of diapause for multiparous *C. opilio* from the EBS may be influenced by the previous thermal history of the clutch during early development.

Comparable to *C. opilio* from the EBS and GSL, the high-latitude majid *Hyas araneus* from the North Sea switches from a one-year to a two-year cycle of embryo incubation at cooler (6 vs. 12 °C) temperatures, due to a long (>11 m) period of diapause in embryo development (Petersen 1995). These diapause periods are hypothesized to time larval release with periods of primary production which vary seasonally at high latitudes (Petersen and Anger, 1997; Petersen, 1995). In the EBS, larval survival and

recruitment success of *C. opilio* may be related to the maximum extent of sea ice in April and highest in years with a strong ice edge bloom (Somerton, 1982). Diapause periods may affect larval survival by changing the timing of larval release in relation to periods of primary production in the EBS. Periods of diapause observed in our study at intermediate temperatures (3 and 1° C) delayed larval release into March and April, respectively; which could serve to postpone larval release into periods of potential increased productivity associated with ice edge retreat. Diapause periods were not observed in clutches incubated at 6° C, potentially because this temperature is outside the range likely to be experienced by ovigerous females in the EBS.

Field observations indicate that significant hatching of *C. opilio* occurs in the EBS by mid-April (Incze *et al.*, 1982) and *C. opilio* zoeae are present in the water column into July (Somerton, 1982). Our study confirms these field observations, as the clutches of multiparous females from the EBS held at 3, 1, and 0° C in hatched in March, April, and May respectively. Hatch timing at different temperatures was similar between multiparous *C. opilio* from the EBS and GSL. In the GSL hatching was observed in April for multiparous females held at 1.85° C for the duration of embryonic development (Moriyasu and Lanteigne, 1998).

Little is known about the effect of embryonic incubation temperature on the duration of larval release for crustaceans at high latitudes. The high-latitude, cold-water, decapods *Paralomis granulosa* and *Lithodes santolla* from Argentina release small numbers of larvae over a period varying from several weeks to two months (Thatje *et al.*, 2003). The duration of hatching (7 to 17 days) in our study was less than that observed

for the high latitude, cold water lithodids, *P. granulosa* and *L. santolla*, with hatching durations of 13 to 61 and 35 to 41 days for females held at 6 ± 0.5° C (Thatje *et al.*, 2003). We did not observe significant differences in the duration of hatching with temperature for *C. opilio* (Figure 1.5).

Successful extrusion of a new clutch within two weeks of the completion of larval release differed among temperature treatments in this study (0% at 6° C, 86% at 3° C, and 100% at 0° C). Reduced spawning success at 6° C may be due to differences in ovarian development rates. After six to seven months at a temperature near their maximum energetic tolerance (Foyle *et al.*, 1989) females may have had fewer resources available for ovarian development which could have either delayed or prevented development of the subsequent clutch. Higher extrusion rates were observed at temperatures (3 and 0° C) similar to those likely to be experienced *in situ* by females in the EBS. These temperatures are likely within their range of energetic tolerance, so ovarian maturation and extrusion of a new clutch can occur soon after the completion of larval release.

Further laboratory and field investigation of the timing and duration of embryonic development and thermal history of female *C. opilio* on a two-year reproductive cycle may help elucidate the proportion on a two-year cycle and its effect on the reproductive potential of the EBS population, starting with observation of embryo development from extrusion to hatch. Future studies should also address differences in ovarian maturation and the timing of oviposition with incubation temperature, as successful extrusion of a new clutch was dramatically reduced at the warmest incubation temperature in this study.

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Table 1.1. Embyro development stages for *C. opilio* from the Gulf of St. Lawrence, Canada (Moriyasu and Lanteigne 1998).

1	prefuniculus
2	funiculus
3	cleavage and blastula
4	gastrula
5	lateral ectodermal band
6	prenauplius
7	nauplius
8	maxilliped formation
9	metanauplius
10	late metanauplius
11	eye pigment formation
12	chromataphore formation
13	reduced yolk
14	prehatching

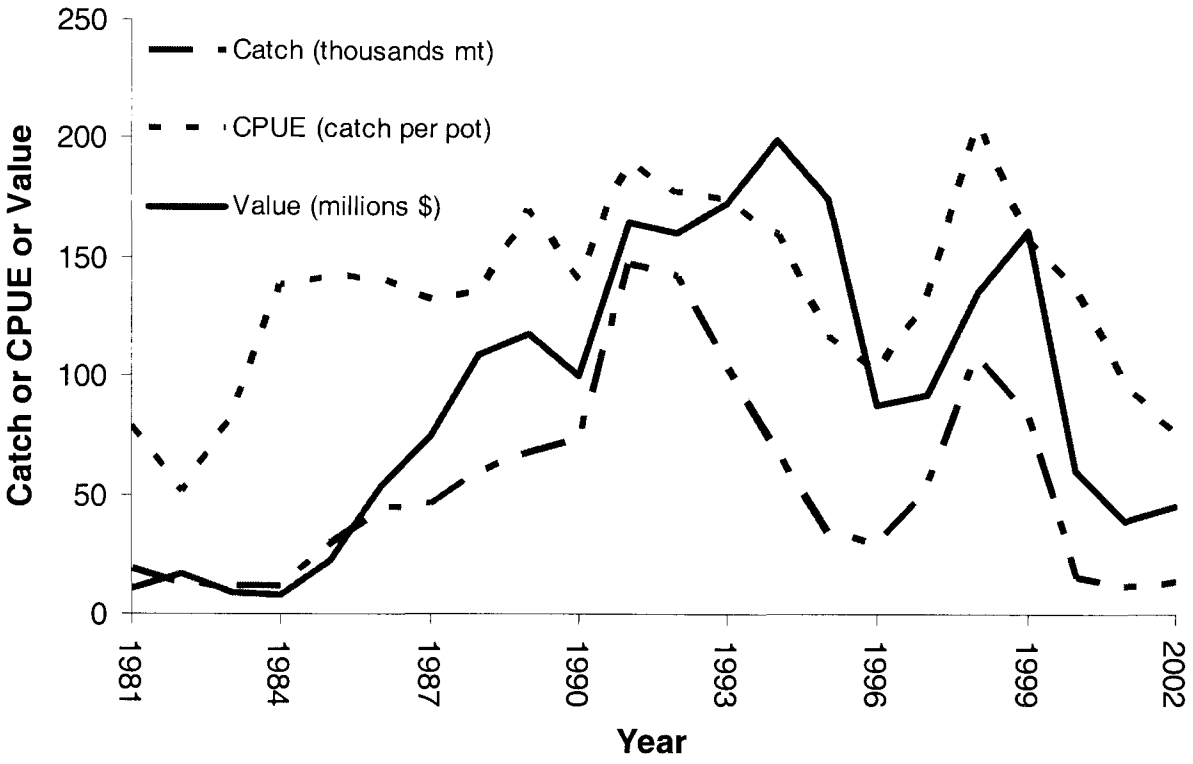

Figure 1.1. Catch, catch per unit effort, and ex-vessel value of the eastern Bering Sea *C. opilio* fishery, 1979-2002.

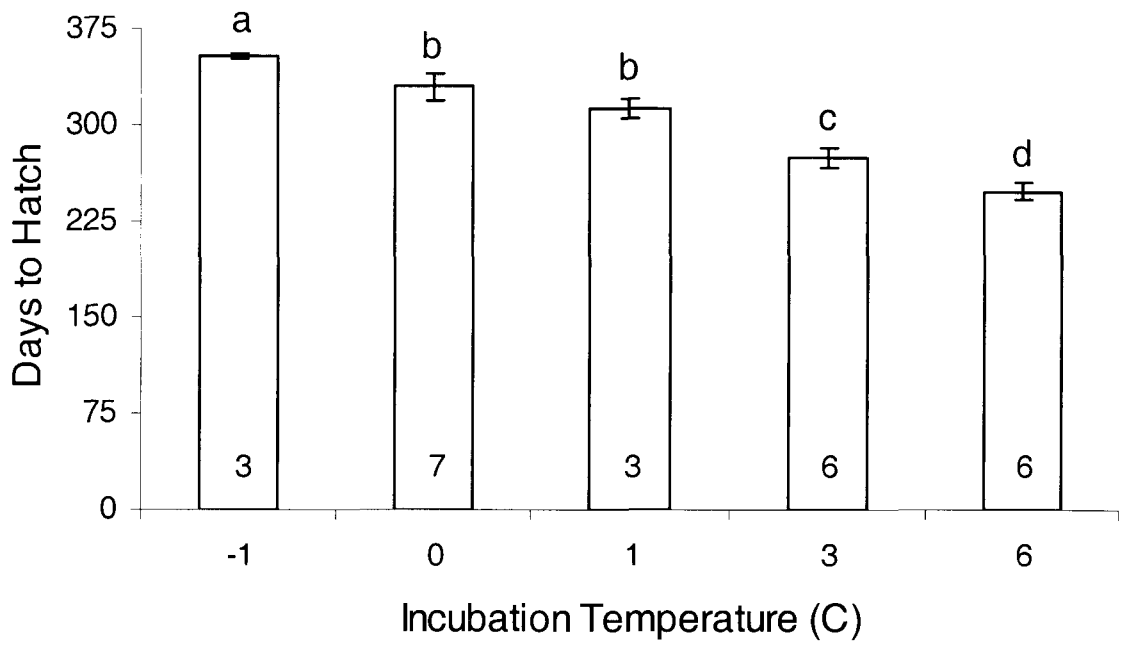

Figure 1.2. Mean duration of embryonic development (d) from collection to hatch for *C. opilio* embryos at constant incubation temperatures. Error bars are ± SD, sample sizes are indicated within the bars, and dissimilar letters indicate significant differences ($p<0.05$).

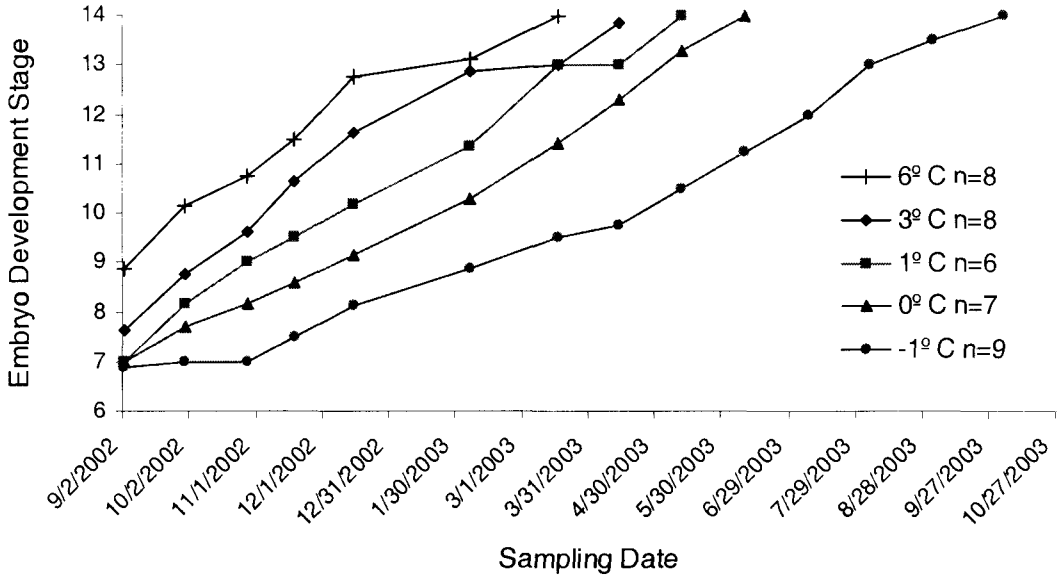

Figure 1.3. Mean stage of embryonic development by sampling date for multiparous *C. opilio* from the eastern Bering Sea held at constant temperature from collection to hatch.

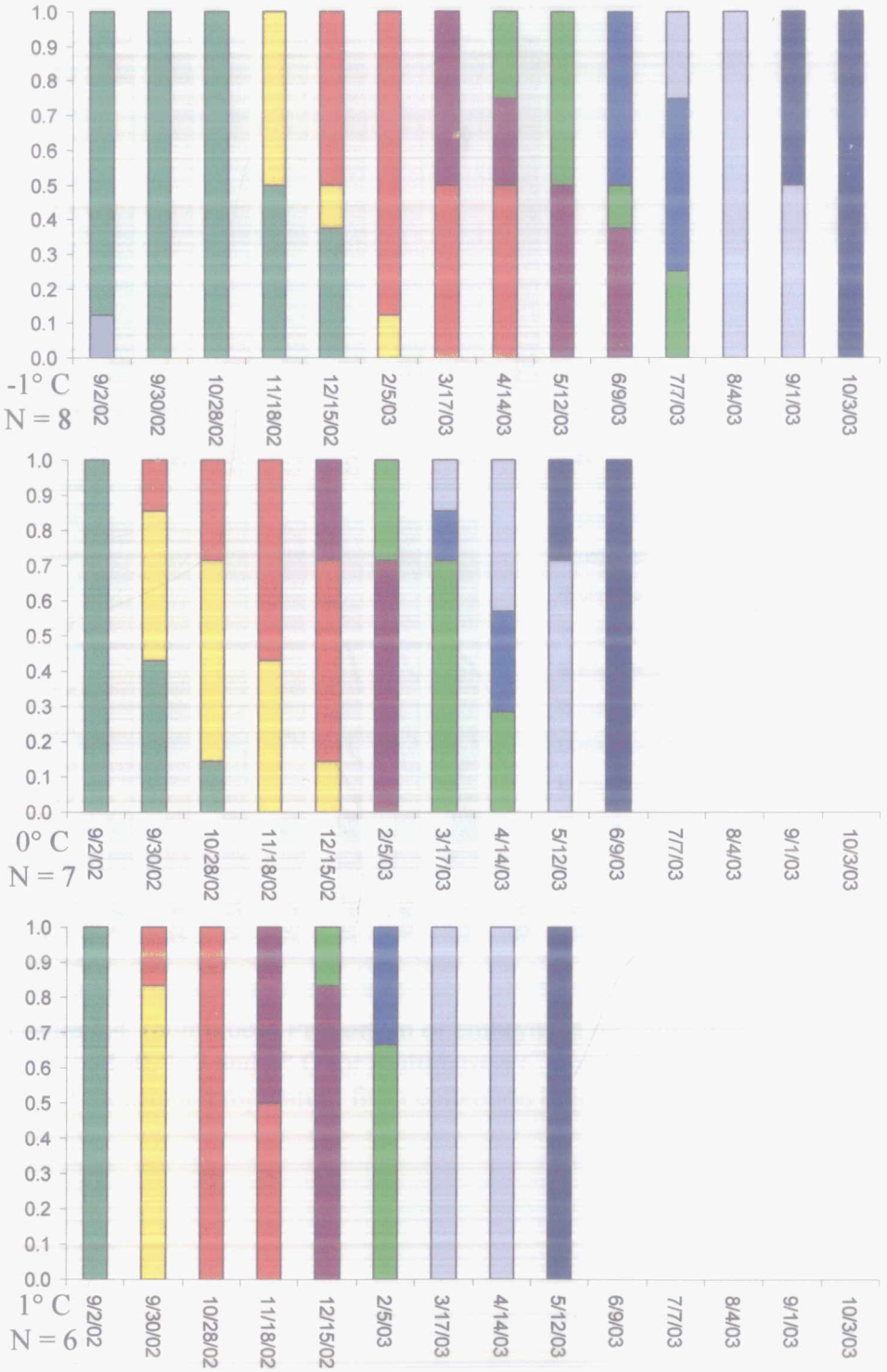

Figure 1.4. Proportion of embryos at developmental stage by sampling date at -1, 0, 1, 3 and 6° C for multiparous *C. opilio* from the eastern Bering Sea held at constant temperature from collection to hatch.

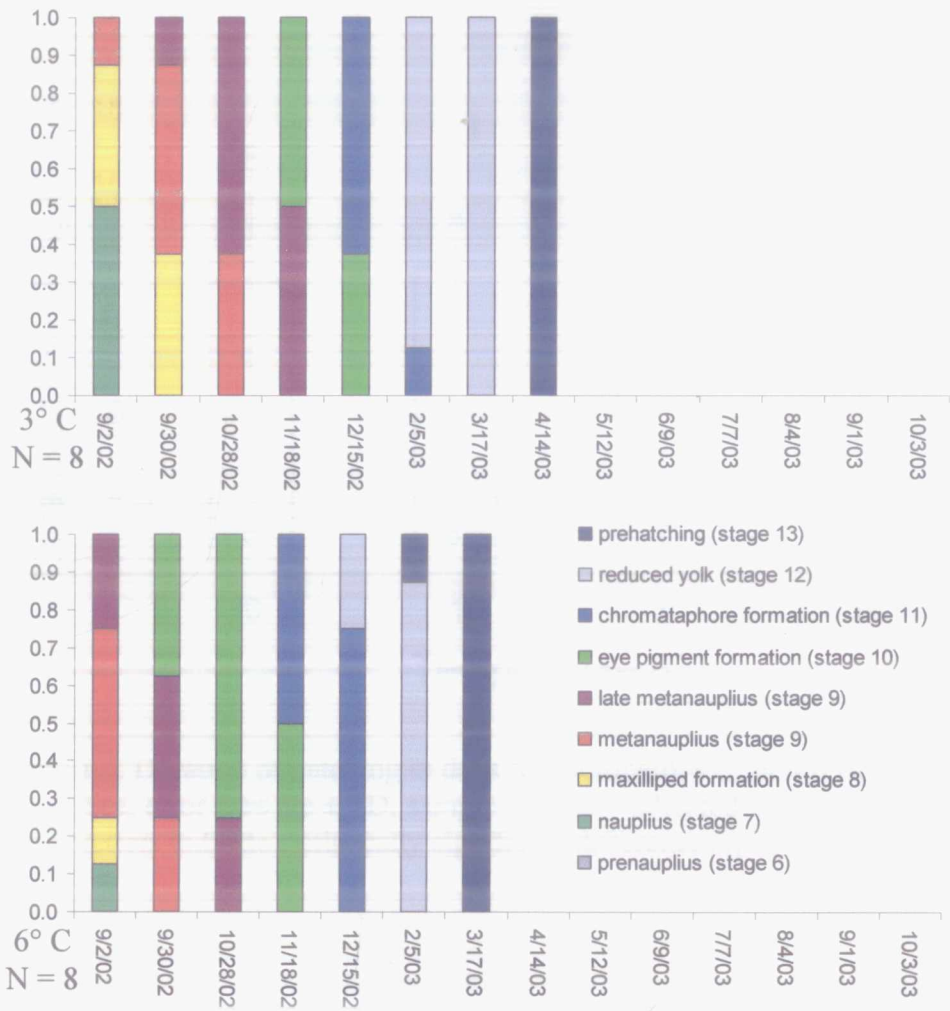

Figure 1.4. (continued) Proportion of embryos at developmental stage by sampling date at -1, 0, 1, 3 and 6° C for multiparous *C. opilio* from the eastern Bering Sea held at constant temperature from collection to hatch.

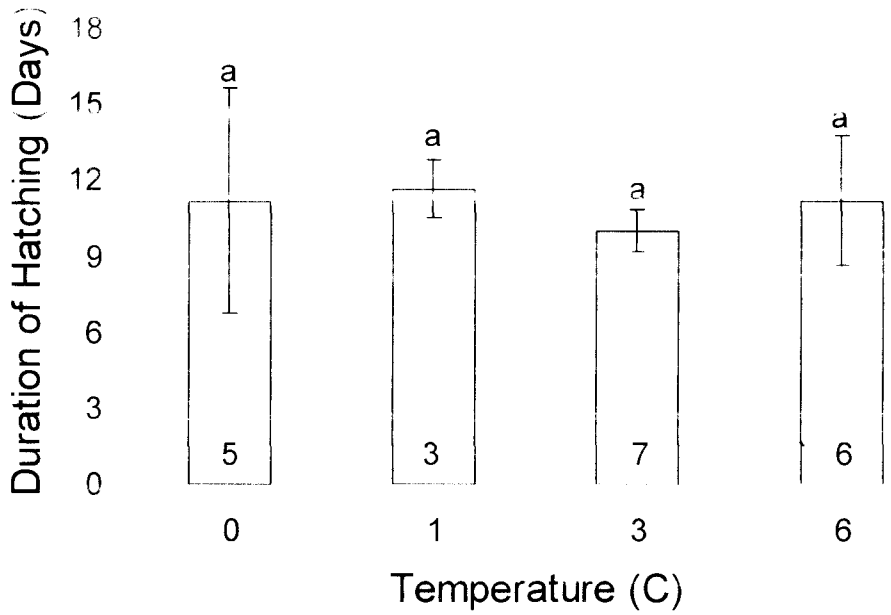

Figure 1.5. Duration of hatching in days for multiparous *C. opilio* from the eastern Bering Sea, error bars are ± SD, sample sizes are indicated within the bars, and dissimilar letters indicate statistically significant differences ($p<0.05$).

Chapter 2.

Changes in zoeae of the snow crab, *Chionoecetes opilio*, with variation in incubation temperature[2]

[2] Joel B. Webb, Ginny L. Eckert, Thomas C. Shirley, and Sherry L. Tamone. Journal of Experimental Marine Biology and Ecology. To be submitted.

2.1 Abstract

Recent declines in catch and abundance of the snow crab, Chionoecetes opilio, in the eastern Bering Sea have motivated further research on the life history of this stenothermic, cold-water species. Small changes in temperature (< 2° C) can lengthen the duration of embryonic incubation in C. opilio from one to two years. The consequences of increased in development time are largely unknown but could include changes in reproductive output and quality of offspring. The purpose of this study was to evaluate the effect of embryonic incubation temperature on C. opilio zoeal morphology, energetic content, and individual weight. Ovigerous female C. opilio were collected by trawl from the eastern Bering Sea in the summer of 2002 and were held in the laboratory at five temperature treatments (-1, 0, 1, 3, and 6° C) from embryo stage five to hatching. The mean duration of incubation was 113 days (32%) longer at -1 than 6 °C. No relationship between larval weight and caloric content with incubation temperature was detected, indicating that increased development time at cooler temperatures may not have an energetic cost. However, several morphological features varied with temperature. Most larval characters including rostro-dorsal length, rostral spine length, lateral spine length, and carapace width were smaller at the warmest incubation temperature. Protopodite length did not differ with temperature. The length of the 3rd abdominal somite consistently increased with increased temperature and may serve as a suitable indicator that could be used in field populations to infer temperature of incubation. Variation in morphology with temperature could confound identification of C. opilio

zoeae from those of a congener, Chionoecetes bairdi because identification of C. bairdi is based on the length of the 3rd abdominal somite.

2.2 Introduction

Snow crabs, Chionoecetes opilio (Fabricius, 1788) (Brachyura: Majidae), are found at high latitudes in cold-water, continental shelf habitats throughout the northern hemisphere. C. opilio are important commercial fisheries throughout their range. However, in the eastern Bering Sea (EBS) catch and catch per unit effort have recently declined (Figure 2.1), and this stock is now considered overfished under the guidelines of the Magnuson-Stevens Fishery Conservation and Management Act (National Marine Fisheries Service 1999).

Chionoecetes opilio is a stenothermic organism, and temperature affects its biology throughout its life history, from embryo to adult. Small changes in temperature (< 2° C) can increase the duration of egg incubation for C. opilio by a full year (Moriyasu and Lanteigne, 1998). In the Gulf of St. Lawrence, Canada, primiparous and multiparous females have a reproductive cycle of approximately 18 and 12 months, respectively at 3 to 5° C (Comeau et al., 1999; Moriyasu and Lanteigne, 1998; Sainte-Marie, 1993; Watson, 1970) and 27 and 24 months, respectively, at -1 to +1° C (Comeau et al. 1999; Moriyasu and Lanteigne 1998; Sainte-Marie 1993). The proportion of crabs on a two-year versus one-year cycle of embryo incubation in the Gulf of St. Lawrence is unknown and may fluctuate with changes in environmental temperature (Moriyasu and Lanteigne, 1998). The two-year cycle from extrusion to hatch is one of the longest known durations of embryonic development for marine invertebrates (Petersen, 1995; Strathmann, 1987).

Field studies in 2003 and 2004 confirmed the presence of both one and two-year reproductive cycles in the EBS with both multiparous and primiparous females on a two-year reproductive cycle at temperatures below 1° C (L. Rugulo NOAA, NMFS, Kodiak, Alaska pers. comm.). Juvenile C. opilio are cold-limited and are found at highest densities in the wild in 0 to 1° C water (Dionne et al., 2003). In laboratory studies, temperature is more important than substrate in determining juvenile distribution, and temperature preferences vary ontogenetically in early benthic instars, with instar III juveniles preferring 0 - 1.5° C temperature strata, and instar V juveniles preferring warmer temperatures of 1.5 - 2.5° C (Dionne et al., 2003).

Adults C. opilio are energetically confined to waters below <7° C, and above this temperature energetic demands exceed metabolic capability. Peak activity (movement) occurs at 0° C and decreases with increasing temperature (Foyle et al., 1989). Female C. opilio undergo a terminal molt to reproductive maturity and subsequently mate without molting. From the puberty molt onward, females undergo an ontogenetic migration following gradients in near bottom temperature or depth from northeast to southwest in the EBS (Ernst et al., 2005; Zheng et al., 2001). The geographic distribution of mature female C. opilio in the EBS has changed in the past two decades, moving northward with a six year lag after the northward contraction of the 2° C near bottom temperature isotherm during the 1975-1979 warming period (Orensanz et al., in press 2005)

Growth, morphology, metabolism, and survival of crustacean larvae are influenced by temperature (Smith et al., 2003; Sulkin and McKeen, 1996; Shirley et al., 1987). Lateral spines of Dungeness crab zoeae reared in the laboratory were 29% longer

in zoeae incubated at 5° C than those at 15° C, and the duration of embryonic incubation was 118 days longer (42 days at 15° C vs. 160 days at 5°C) (Shirley et al., 1987). Longer spines may enhance defenses against predation during an extended planktonic period (Shirley et al., 1987). Similar patterns of variation are found in larval C. opilio; zoeae from embryos reared at 5° C had larger carapace widths than those reared at 8 or 10 ° C (Takeda et al., 1992). Morphometric measurements for zoea I C. opilio vary geographically (Davidson and Chin, 1991; Wencker et al., 1982), and allometric spine growth caused by temperature could confound the characteristics used to separate C. opilio zoeae from those of C. bairdi. Identification of Chionoecetes zoeae is based on differences in the length and orientation of dorsal, lateral, and abdominal spines (Wencker et al., 1982; Haynes, 1973). Hatching of the southern spiny lobster, Jasus edwardsii, was delayed two months incubated in cold (10.5° C) versus warm (18° C) temperatures (Smith et al., 2003). Phyllosoma larvae from the ambient treatment (9.5 to 13° C) were larger, and ascorbic acid and some lipid class levels were negatively correlated with increasing incubation temperature (Smith et al., 2003). Larval survival also varies with incubation temperature. Post-hatch larvae of the paleamonid prawn, Macrobranchium rosenbergii, incubated at 25° C had greater survival than those incubated at 29 or 31° C across a range of salinities (Gomez-Diaz, 1987). Zoeae of the high latitude, cold water, lithodid, Paralomis granulosa, reared at temperatures of 1, 3, 6, 9, 12, and 15° C had maximum survival rates at 6 and 9° C. Zoeae completed development through metamorphosis, with the exception of the 1°C treatment in which

52% of zoeae survived to megalopae but none survived through metamorphosis (Anger et al. 2003)

Planktonic life has long been considered a major source of mortality in marine invertebrate populations (reviewed by Pechenik, 1999; Morgan, 1995; Rumrill, 1990). If varying incubation temperatures significantly affect the quality of C. opilio zoeae, then incubation temperature could affect recruitment and population dynamics of the population. While numerous studies have examined the size (Davidson and Chin, 1991 Pohle, 1991; Wencker et al., 1982) duration (Davidson and Chin, 1991; Wencker et al., 1982), nutrition (Kon, 1979; Lovrich and Oullet, 1994; Motoh, 1982), morphology (Haynes, 1981, 1973; Pohle, 1991; Wencker et al., 1982) and growth (Incze et al., 1984; Lovrich and Oullet, 1994) of C. opilio zoeae in the field and laboratory the effect of varying incubation temperature on zoeal quality has not been examined. The present study investigated the effect of incubation temperature on individual dry weights, energy content, and morphology of C. opilio zoeae hatched in the laboratory from ovigerous females collected in the EBS.

2.3 Methods and Materials

Ovigerous females were collected by bottom trawl in July 2002 from south of St. Matthew Island (58° N, 193° W) in the EBS at a depth of 117 m and a bottom temperature of 3.2° C and transported to Juneau by air on July 10, 2002. Twenty-five females per tank were maintained in 700 L flow-through seawater tanks cooled by chillers (Frigid Units™ Inc. Model D1-33) to temperatures of 6, 3, 1, 0, and -1° C until their embryos hatched. Temperatures were monitored every 15 min using Stowaway

Tidbit™ temperature loggers (Onset Computer Inc.). Mean tank temperatures °C (± 1SD) averaged daily over the duration of the study for the 6, 3, 1 0 and -1 °C treatments were 6.32 (±0.36), 3.24 (±0.18), 1.37 (±1.03), 0.12 (± 0.94), and -0.89 (± 0.59), respectively. Females were assigned to temperature treatments in a random stratified design based on carapace width. Only multiparous females with full clutches and less than two missing or damaged limbs were used in the study. Embryos were collected monthly from each female and the developmental stage of the clutch was assessed (Moriyasu and Lanteigne, 1998) with a Leica model MZ 8 dissecting microscope. When embryos reached the final developmental stage, seven females with a representative range of carapace widths were selected from each treatment and placed in plastic containers (33 x 18 x 10 cm) with 500 µm (47% open) Nitex™ mesh on two sides to retain hatching zoeae and allow water exchange, while in the tank. Females were monitored daily to detect hatching, and hatched zoeae were collected daily by reverse filtration (45 µm) and sorted into pre- or stage-one zoeae. Time from collection to hatch increased with decreasing temperatures lasting (mean days ± SD) 240 ± 10 d at 6° C, 276 ± 8 d at 3° C, 314 ± 8 d at 1° C, 331 ± 11 d at 0° C, and 353 ± 2 d at -1° C. (see Chapter 1 for further details). All statistical analyses were conducted using SAS release 8.02 (SAS Institute, Cary, NC).

2.3.1 Morphology

Stage-one zoeae were collected for morphological measurements and preserved in 5% buffered formalin. Rostro-dorsal length (RDL), dorsal spine length (DSL), rostral spine length (RSL), carapace width (CW), lateral spine length (LSL), protopodite length

(PL), and the length of the posterior spinous lateral process of the third abdominal somite (3^{rd} PLS) (Figure 2.2) were measured to the nearest 0.01 mm using an ocular micrometer on a Leica model MZ8 dissecting microscope at 20, 32, or 50x magnifications. Three zoeae from four females (n=12) were measured for treatments in which zoeae successfully hatched. Sample sizes were limited at 1° and 0° C because of low zoeal survival past the prezoeal stage, and insufficient numbers of zoeae (<100 per day) hatched in the -1° C treatment for inclusion in analysis. Morphological characters were compared among temperatures using one-way ANOVA and Tukey-Kramer multiple comparisons test (Glantz and Slinker, 2001).

2.3.2 Individual Weights

After collection, 20 stage-one zoeae from each female with no visible damage were rinsed with freshwater, towel-dried, placed individually in 0.1 ml centrifuge vials, and freeze-dried for at least 24 hours. Individual weights of freeze-dried zoeae were measured to the nearest 0.1 μg with a Cahn model C15 ultramicrobalance. The Welch ANOVA for unequal variance was used to compare mean dry weights were compared between temperature (Glantz and Slinker 2001), and post-hoc multiple comparisons were made with the Tukey-Kramer test.

2.3.3 Energetics

When sufficient numbers of zoeae (>1000) hatched during a 24 h period, a sample consisting of 500 stage-one zoeae was collected from each female and dried to constant weight in a 45° C oven for at least 24 hours, compressed into a pellet, and weighed to the nearest 0.1 mg. Pellets had a mean weight (± 1 SD) of 0.040 ± 0.003 mg. Energetic

content was measured using a Parr™ model 1425 semi-microbomb calorimeter. Sample sizes were limited in the coldest treatments (-1, 0, and 1° C) because of low zoeal survival past the prezoeal stage and few females hatching adequate numbers of zoeae in a twenty-four hour period. Statistical comparisons between temperatures were made with one-way ANOVA and Tukey-Kramer multiple comparisons tests; data from the 0 and 1° C treatment was excluded from analysis due to low sample size.

2.4 Results

2.4.1 Morphology

Except for PL, larval morphology differed significantly among incubation temperatures (Table 2.1). Temperature explained 76% of the variation in length of the 3rd PLS, 69% of DSL, 61% of RDL, 44% of LSL, 43% of CW, 39% of RSL, and 11% of PL. Zoeae incubated at 6° C were significantly smaller in RDL and RSL than those from other temperatures (Table 2.2). Dorsal spine length was greatest in zoeae incubated at 3° C; CW and LSL were significantly greater in 3° C than 6° C incubated zoeae. The PLS of the third abdominal segment increased in length with increasing incubation temperature (Table 2.2).

2.4.2 Individual Weights

The weights (mean ± 1SD) of post-hatch zoeae incubated at 6° C (81.2 μg ± 7.8, n=47) were significantly heavier ($F_{4,99} = 5.48$, $p<0.0005$) than those incubated at 1° C (71.2 μg ± 5.8, n=15) and 0° C (75.1 μg ± 6.2 n=18). However, the weights of zoeae incubated at 3° (76.3 μg ± 4.0, n=18) and -1° C (77.1 μg ± 8.4, n=16) were not significantly different than those incubated at 6° C.

2.4.3 Energetics

No significant differences in energetic content ($F_{1, 15} = 0.10$, $p<0.7526$) with temperature were observed. The energy content (mean ± 1SD) of zoeae at 6° C was 2854 ± 392 cal/g (n=9) which was not significantly different than 2801 ± 82 cal/g (n=6) at 3° C. The mean energy content of zoeae was at 2812 cal/g (n=2) at 1° C.

2.5 Discussion

Phenotypic plasticity has been observed in marine invertebrates in response to temperature (Smith et al., 2003), predator cues (Harvell 1992), maternal provisioning (Reitzel and Heyland, 2001), and food availability (Reitzel et al., 2004). Significant plasticity in larval size with temperature was observed in this study and was consistent with observations of longer spines at lower incubation temperatures for other species (Smith et al., 2003; Takeda et al., 1992; Shirley et al., 1987). One unique observation from this study was the observed increase in the length of the 3rd PLS with increasing incubation temperature. The length of the 3rd PLS responded in the opposite direction as other morphological characters and was also more sensitive to incubation temperature than any other morphological measurement.

Differentiation of stage-one C. opilio zoeae from Tanner crab, C. bairdi, zoeae is based on small differences in morphology and spine length (Wencker et al., 1982; Haynes, 1973). Chionoecetes bairdi overlaps C. opilio in geographic distribution, and therefore a way to distinguish their larvae is needed. An early comparative study (Haynes, 1973) using zoeae of known parentage determined that the most useful character for identifying zoeae of each species was the length of the posterior lateral

spines (PLS) of the third or fourth abdominal segments. For C. bairdi, the PLS overlap the adjacent segments by about one-third of the length of the spines. In stage-one zoeae of C. opilio, the PLS of the third abdominal segment barely extend past the posterior margin of the fourth segment, and the PLS of the fourth abdominal segment do not reach the posterior margin of the fifth segment (Haynes, 1973). Wencker et al. (1982), using field collected and laboratory reared zoeal samples, concluded that the PLS length characteristic could not clearly differentiate between species. Instead RDL, carapace lateral spine length and orientation, and the relationship of the length of the lateral process of the 3rd abdominal somite to the length of the posterior margin of the same segment were used as indicators to distinguish between species (Wencker et al., 1982). Our study demonstrates that 3rd PLS length is temperature dependent and may not be a reliable character for species identification. Water temperature, though interannually variable, generally increases with decreasing latitude in the EBS (Luchin et al., 1999, 2002). Because PLS length is sensitive to incubation temperature, a latitudinal gradient in PLS length might be observed in field collections due to increasing incubation temperatures. Such temporal and spatial variation would confound the identification of Chionoecetes zoeae based on the characters proposed by Haynes (1973). Only one other study has reported 3rd PLS lengths for C. opilio zoeae (Pohle, 1991), making broad comparisons of this character difficult. The 3rd PLS lengths reported in Pohle (1991) for C. opilio from the Gulf of St. Lawrence correspond with those observed in our 3° C treatment, and though duration and temperature of incubation were not reported by Pohle (1991), temperature at hatch was 3° C. Further investigation of larval morphology,

particularly 3rd PLS length in field-collected zoeae from the EBS may provide insights into the thermal history of the clutch and indicate the approximate geographic origin of C. opilio zoeae.

Zoeae from this study were intermediate in size compared to published morphological descriptions of conspecifics from the northwestern Atlantic (Davidson and Chin, 1991; Pohle, 1991), the northern Pacific (Wencker et al., 1982; Haynes, 1973) and the Sea of Japan (Motoh, 1980) (Table 2.3). Chionoecetes opilio zoeae from the Sea of Japan (Motoh, 1973) were larger than those from any other study. Zoeae from our study were larger in mean size than those from those described by Haynes (1973) from the EBS but smaller in size than those collected from the EBS in a separate study (Wencker et. al., 1982) (Table 2.3). Though not observed in this study, variation in zoeal size may be due to differences in environmental factors with area and time.

Variation in patterns of spine growth with incubation temperature may be attributable to other adaptive constraints on zoeal morphology such as buoyancy, sinking rate, swimming efficiency, and defense against predation (Anger, 2001; Morgan, 1995). Spines which extend outward from the body (dorsal, rostral, lateral, and protopodite) make greater contributions to zoeal size than the 3rd PLS and, therefore, may be more constrained. Morphological differences between zoeae of estuarine and coastal crab species from the southeastern United States presumably were related to differences in predation pressure (Morgan 1990). Estuarine zoeae have conserved a body plan with prominent defensive spines as protection from increased predation by planktivorous fishes, which are more abundant in estuaries than in coastal waters. Zoeae of crab

species exported to coastal waters have larger body sizes and shorter spines than their estuarine counterparts (Morgan, 1989, 1990). Chionoecetes opilio zoeae are morphologically similar in terms of spination to estuarine zoeae rather than larvae exported into coastal waters. Selective pressure from predation could hypothetically conserve a body plan with greater defensive capability in C. opilio zoeae.

Clear trends in zoeal weight with temperature were not observed in this study. Although zoeae incubated at 1° C weighed significantly less than those incubated at 6° C, zoeal weights at -1° and 0°, and 3° C were not significantly different than those at 6° C. Zoeae in our study had an overall mean weight of 78.0 µg. Previously reported mean individual dry weights of post-hatch zoeae from two primiparous females incubated at ~10° C from the northwestern Atlantic were 62 µg and 70 µg (Lovrich and Oullet, 1994). Mean weights of zoeae from individual adult females in our study ranged from 68.3 µg to 84.5 µg. The wider range of zoeal weights observed in this study compared to Lovrich and Oullet (1994) could be due a result of larger sample sizes or to differences in maternal reproductive status in multiparous compared to primiparous females.

The distribution and movement of mature female C. opilio in the EBS may be associated with near-bottom water temperatures below 2°C; and larvae hatched are likely to be retained in the northern portion of the EBS (Ernst et al., 2005; Orensanz et al., in press 2005). According to our results, incubation of embryos at 2° C is unlikely to have significant positive or negative effects on larval quality (energy content or individual weight). Based on temperature records from NMFS summer trawl surveys, the previous thermal history of females collected for this study was likely ~ 0 to 3°C and was 3°C at

the time of collection. In this temperature range the length of embryonic incubation occurring in situ would be approximately two to three months since a new clutch is extruded soon after larval release, which occurs in April in the EBS (Incze et al. 1982). Because females collected for this study were on a one-year reproductive cycle (see chapter 1), this period of time in situ would most likely have little affect on the results of this study, earlier exposure to differing incubation temperatures would be expected to shorten the duration of incubation at 6 °C, increase the duration of incubation at 1, 0, and -1 °C, and have little effect at 3° C. Incubation at experimental temperatures from extrusion to hatch would be expected to increase the significance of differences in zoeal morphology between temperatures due to the greater differences in development time among temperatures. Specifically, differences in 3rd PLS length could be increased due to longer durations of incubation at 1, 0, and -1°C.

In conclusion, significant differences in most morphological characteristics of C. opilio zoeae were related to incubation temperature in our study. Conversely, clear trends in the quality of post-hatch C. opilio zoeae with incubation temperature were not evident. Detailed analysis of larval biochemistry (e.g. Smith et. al., 2003) is recommended in future studies as a method for evaluating the possible costs of increased development time at lower temperatures. Conducting a comparative study of the quality and survival of zoeae from both one-year and two-year reproductive cycles might further clarify the costs of extended periods of embryonic development and potential changes in the quality of post-hatch zoeae.

2.6 Acknowledgements

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Table 2.1. ANOVA results for morphological differences of stage-one, C. opilio zoeae, incubated at 0, 1, 3, and 6° C.

Character	DF, Error DF	F	P-value
Rostro-dorsal length	3,41	22.85	<0.0001
Dorsal spine	3,41	27.70	<0.0001
Rostral spine	3,41	9.77	<0.0001
Protopodite length	3,41	1.70	<0.1815
Carapace width	3,41	5.76	<0.0022
Lateral spine	3,41	7.36	<0.0005
3rd posterior lateral spine	3,41	38.19	<0.0001

Table 2.2. Mean +/- SD spine lengths (mm) for stage-one, C. opilio zoeae from different embryonic incubation temperatures, dissimilar characters indicate significant differences among treatments for each spine length ($p<0.05$). Larval characters are RDL, rostro-dorsal length or total length; DSL, dorsal spine length; RSL, rostral spine length; CW, carapace width; LSL, lateral spine length; PL, Protopodite Length; and 3rd PLS, length of the posterior lateral spine of the third abdominal segment.

	Incubation Temperature (°C)			
Larval Character	**0**	**1**	**3**	**6**
N=	9	6	15	15
RDL	4.46 +/- 0.22 a	4.55 +/- 0.16 ab	4.66 +/- 0.12 b	4.32 +/- 0.09 c
DSL	1.92 +/- 0.12 a	1.95 +/- 0.07 a	2.07 +/- 0.05 b	1.88 +/- 0.06 a
RSL	1.69 +/- 0.04 a	1.70 +/- 0.06 a	1.69 +/- 0.09 a	1.58 +/- 0.05 b
CW	2.92 +/- 0.05 ab	2.89 +/- 0.04 ab	3.00 +/- 0.08 a	2.85 +/- 0.10 b
LSL	0.85 +/- 0.03 ab	0.85 +/- 0.02 ab	0.90 +/- 0.06 a	0.81 +/- 0.03 b
PL	1.50 +/- 0.04 a	1.49 +/- 0.07 a	1.54 +/- 0.06 a	1.49 +/- 0.08 a
3rd PLS	0.31 +/- 0.02 a	0.35 +/- 0.02 ab	0.37 +/- 0.04 b	0.45 +/- 0.02 c

Table 2.3. Comparison of published morphological descriptions of stage-one C. opilio zoeae. Missing values were unavailable from manuscripts.

Author	This Study	a	b	c	d	e
Region	Eastern Bering Sea	Gulf of St. Lawrence, Canada	Cape Breton, Canada	Eastern Bering Sea	Sea of Japan	Bristol Bay, Alaska
N=	61	50				50
Measurement	Mean +/- SD	Mean +/- SD	Mean (Range)	Range	Range	Mean (Range)
Rostro-dorsal	4.42 +/- 0.21	4.92 +/- 0.17	4.84 (4.68-5.00)	4.5-5.4	4.85-5.52	4.17 (3.96-4.55)
Dorsal	1.93 +/- 0.10	2.12 +/- 0.13	1.96 (1.80-2.20)			
Rostral	1.63 +/- 0.11	1.72 +/- 0.10	1.68 (1.48-1.88)			
Carapace Width	2.87 +/- 0.14	2.75 +/- 0.20	3.22 (2.96-3.70)		3.30-3.98	2.73 (2.52-2.97)
Lateral	0.84 +/- 0.06	0.96 +/- 0.07	0.84 (0.74-0.94)			
Protopodite	1.49 +/- 0.09	1.81 +/- 0.09	1.72 (1.56-2.00)			
3rd PLS	0.40 +/- 0.06	0.37 +/- 0.04				

Published sources: a – Pohle (1991), b- Davidson and Chin (1991), c – Wencker et al. (1982), d- Motoh (1973), e – Haynes (1973)

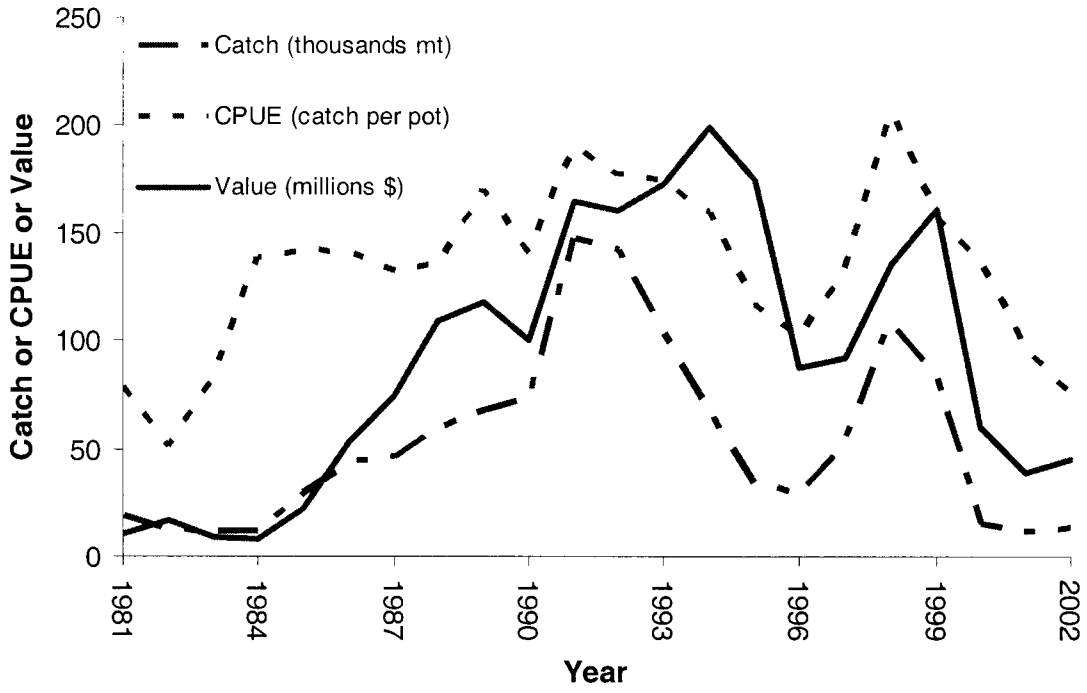

Figure 2.1 Catch, catch per unit effort, and value of the eastern Bering Sea *C. opilio* fishery,1979-2002 (NMFS).

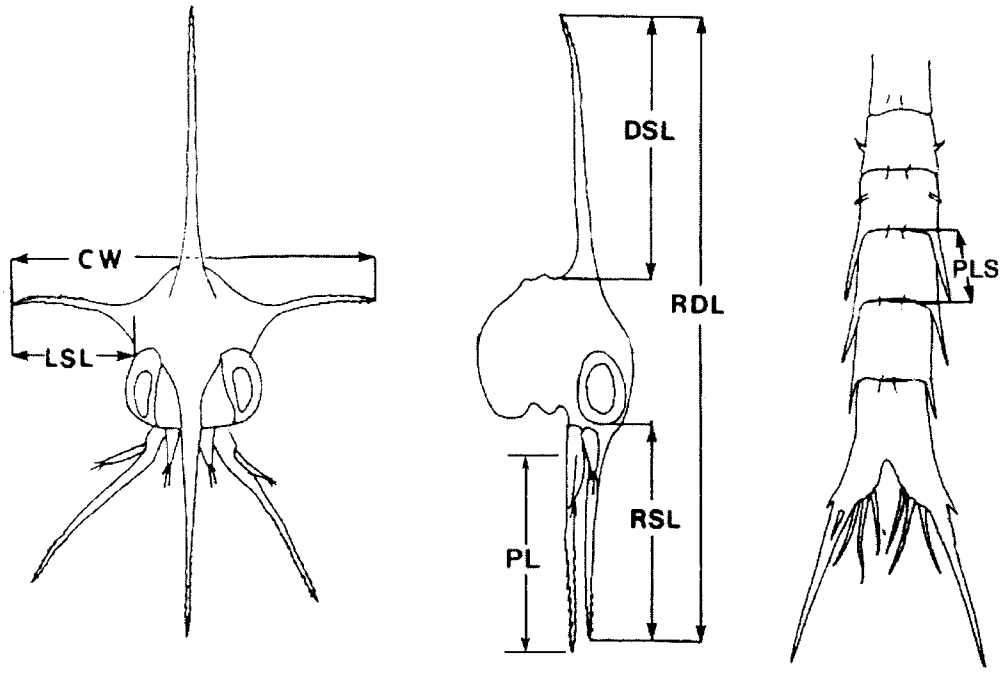

Figure 2.2. (adapted from Pohle 1991) Morphologic measurements used for determining variation with temperature for stage-one *C. opilio* zoea. CW, carapace width; LSL, lateral spine length; DSL, dorsal spine length; RDL, rostro-dorsal length or total length; RSL, rostral spine length; PL, Protopodite Length; PLS, posterior lateral spine length.

General Conclusions:

A targeted fishery for the snow crab (*Chionoecetes opilio*) has occurred in the EBS for approximately 30 years. After peaking in the early 1990s, catches have dropped to historically low levels and have remained low for the past five years (Bowers 2004). The stock was declared "overfished" in 1999 under the Magnuson-Stevens Fishery Conservation and Management Act and is currently under a rebuilding plan (National Marine Fisheries Service 1999).

The Bering Sea ecosystem has changed dramatically since a climatic regime shift in 1976-1977 that resulted in warmer water temperatures and an associated northward shift in the biogeographical distribution of cold water organisms including *C. opilio* (Orensanz et al. in press 2005) and changes in ecosystem dynamics (Overland et al. 2005, Hunt et al. 2002). This warming trend may continue with further effects on the eastern Bering Sea ecosystem (Overland and Stabeno 2004). *Chionoecetes opilio* are stenothermic with small changes temperature affecting the species throughout their life history (Ernst et al. 2005, Dionne et al. 2003, Moriyasu and Lanteigne 1998, St. Marie and Gilbert 1998, Foyle et al. 1989). Notably, small changes in ambient temperature can extend the time required for embryonic development from one to two years (Rugolo 2004, Moriyasu and Lanteigne 1998).

We evaluated the effect of incubation temperature on snow crab from the EBS by holding multiparous females at five discrete temperatures (-1, 0, 1, 3, and 6° C) from collection (approximately three months after extrusion) to at least two weeks after the completion of larval release. Our results give some important insights into the effect of

temperature on the early life history stages of *C. opilio*, which could be important for understanding potential responses of the EBS population to continued ecosystem change. This project was divided into two sections. Embryonic development, hatching, and spawning were addressed in Chapter 1, and the characteristics of post hatch zoeae reared at different incubation temperatures were covered in Chapter 2.

Patterns of embryonic development and differences in the duration of embryonic development in this study were similar to those of conspecifics from the GSL on a one-year reproductive cycle (Moriyasu and Lanteigne 1998). Cold temperatures did not extend embryonic incubation to two years in our study, so it is likely that a one or two year requirement for embryonic development is determined early in embryonic development. Female *C. opilio* in the EBS undergo an ontogenetic migration from colder to warmer waters with the greatest distance covered after the primiparous molt to reproductive maturity (Ernst et al. 2005). This migration into warmer water may be important in maintaining a higher proportion of the EBS *C. opilio* population on a one versus two year cycle of embryo incubation, especially if clutches are at warmer temperatures during the first several months after extrusion.

The duration of incubation increased with decreasing temperature by about 17 d per 1° C. This decrease is similar to that observed in *C. opilio* from the Gulf of St. Lawrence (Moriyasu and Lanteigne 1998). However, the timing and duration of diapause differed from Canadian conspecifics, with shorter periods of diapause observed in this study than in multiparous females from the GSL. The presence of absence of a diapause period with temperature may aid in timing larval release with primary production

associated with ice edge retreat, which may increase larval survival (Somerton 1982). The duration of larval release did not vary significantly with incubation temperature. Female *C. opilio* extrude a new clutch of embryos onto the abdomen, often within a few days completion of larval release. No females completing larval release at 6° extruded new clutches, while all females at 0° C and all except one at 3° C had undergone oviposition with two weeks of the end of hatching. Exposure to temperatures in the range of 6° C may inhibit the internal development of the subsequent clutch.

The consequences of differing incubation temperature for post hatch *C. opilio* zoeae were assessed by zoeal weight, energetic content, and morphology. Significant differences in zoeal weight or energy content with incubation temperature were not observed, implying that longer incubation times at colder temperatures may not have increased energetic costs. Zoeal morphology varied significantly with temperature with the greatest differences in the length of the 3rd abdominal somite which increased in length with increasing incubation temperature. Significant increases in the length of the 3rd abdominal somite with decreases in incubation temperature of 1 °C may make this characteristic useful as an indicator of incubation temperature *in situ*. The length of this spine is unlikely to affect zoeal buoyancy, swimming, feeding, or defense. *C. opilio* zoeal morphology is more similar to estuarine zoeae than zoeae which are exported to coastal waters in the Atlantic because they have conserved longer spine lengths for protection from planktivorous fishes (Morgan 1989, 1990). Thermal influences on spine length may be constrained by selection for defensive spination. The length of the 3rd abdominal somite was used by Haynes (1973) to differentiate *C. opilio* and *C. bairdi*

zoeae I in the EBS. Temperature induced variation in the length of this spine makes it an unreliable character and the more comprehensive identification methods of Wencker et al. (1982) are recommended.

The climatic coupling between the northern Pacific Ocean and Bering Sea may have weakened, and the warming trend in the Bering Sea is likely to continue into the near future (Overland et al. 2005). If this is the case, the northward transition of cold-water species in the eastern Bering Sea likely will continue. Our findings indicate by default that the proportion of females on a two-year versus one-year reproductive cycle may be determined during the first three to four months of embryo development. Further research into the distribution and movement of ovigerous females in relation to bottom temperature during this time span would be an important step for managers in projecting the effect of varying durations of embryonic development on the reproductive potential of the population. If water temperatures warm into the range of 6° C in areas of female *C. opilio* distribution in the EBS, larval survival, and the long term reproductive health of female *C. opilio* may be affected. Differences in development time with incubation temperature do not appear to have an energetic cost for *C. opilio* zoeae, but survival of zoeae would be affected by changes in the timing of larval release and peaks in primary production with temperature.

Our results indicate temperatures within the range of 0 to 3° C may be optimal for *C. opilio* reproduction. The timing of embryo development and hatching, energy content and weight of post-hatch zoeae, and successful extrusion of a new clutch were favorable in this temperature range. Warmer (6° C) temperatures may decrease the reproductive

success of *C. opilio* by timing hatch during times of poor food availability and reducing the successful spawning of a subsequent clutch. Mature female mortality was high and the duration of embryonic development extended considerably past the timing observed *in situ* at colder (-1° C) temperatures.

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